

RESEARCH ARTICLE

AMELIORATIVE EFFECT OF *BAMBUSA ARUNDINACEA* AGAINST ADJUVANT ARTHRITIS-WITH SPECIAL REFERENCE TO BONE EROSION AND TROPICAL SPLENOMEGALY*Rathod Jaimik D¹, Pathak Nimish L¹, Patel Ritesh G¹, Jivani Nuruddin P¹, Patel Laxman D¹, Chauhan Vijay²¹PG & Research Dept. of Pharmacology C.U.Shah College of Pharmacy & Research, Wadhwan- 363030, Gujarat, INDIA²Astron Research Center, Ahmadabad, Gujarat, INDIA-380054*Corresponding Author's E-mail: jaimikrathod275@gmail.com

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ABSTRACT

The present investigation was aimed to study the Anti-arthritis activity of *Bambusa arundinacea* in treating Rheumatoid Arthritis (RA) using CFA-induced arthritis animal model. CFA was introduced in female Wistar rats by Sub planter Injection (0.1 ml). These rats were treated daily with oral administration of different doses of Methanolic extract of *B. arundinacea* beginning on the day after the onset of arthritis (till day 12th). During 13th to 21st day period no drug treatment is given to test and standard group animals. On 21st day parameters were observed. In the present study, the effect of *Bambusa arundinacea* Methanolic extract on the Arthritis was studied by analyzing various markers of Bone erosion like histological, radiological analysis of the joints. For evaluation of Anti-arthritis activity other parameters analyzed are Paw volume, Arthritic index, Rheumatoid Factor, Erythrocyte Sedimentation Rate (ESR) and Spleen histopathology. The powdered leaves are used for hot extraction by using methanol as solvent. The Anti-arthritis activity of the dry extracts was performed using female rats of about 200 to 250gms. The Methanolic extract of *Bambusa arundinacea* significantly (dose dependent) decreased the bone erosion, spleen enlargement & rheumatoid factor etc. at a dose (100mg/kg, 200mg/kg, 300mg/kg) compared to the control group but less compared to Standard drug (Dexamethasone 5 mg/kg i.p.).

Key Words: Complete Freund's Adjuvant, Rheumatoid arthritis, Bone Erosion, Spleen Histopathology.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic and chronic inflammatory autoimmune disease, is characterized by synovial lining hyperplasia, chronic synovitis and progressive bone and cartilage damage.^{1,2} Complete Freund's Adjuvant (CFA) contains heat killed Mycobacterium in water-in-oil (paraffin oil) Emulsion. In Arthritis, Bone erosions representing bony destruction were evident on bone unprotected by cartilage. The spleen provided a readily available source of cells known to be involved in arthritis, so splenomegaly (spleen enlargement) is a marker for Presence of Arthritis.³

The therapies available for RA are disease-modifying anti-rheumatic drugs (DMARDs). The uses of DMARDs have of long-term side effects and toxicity, therefore their usage have been impeded.⁴ India is the largest and oldest botanical garden in world. *Bambusa arundinacea* is distributed throughout the moist parts of India. The Methanolic extract showed the presence of flavonoids, glycosides, traces of alkaloids and phytosterols, which give Anti-ulcer & Anti-inflammatory activity.⁵ Quite a large number of studies have found that different parts of *Bambusa arundinacea* possess medicinal activities like Antidiabetic activity,⁶ Antifertility effect,⁷ Antibacterial activity,⁸ Anti-inflammatory and Protective effects.⁹

MATERIALS AND METHODS

1 Animals

Protocol of the study was passed by Institutional Ethics Committee of C. U Shah College of Pharmacy and Research, Wadhwan. The study was carried out with adult

female Wistar rats weighing 200–300 g. Animals were acclimatized to the experimental conditions in cages and kept under standard environmental conditions (22 ± 3°C; 12/12 h light/dark cycle). Rats were allowed to feed and water ad libitum.

2 Plant materials

The disease free fresh plant material (Leaves) was collected in the month of December 2011 from surrounding area of Ahmedabad, Gujarat and authenticated at Botany Department of Gujarat University by Dr. H. A. Solanki. After authentication, fresh leaves were collected in bulk from the tree, shade dried, pulverized and passed through sieve no.40 to obtain coarse powder.

3 Preparation of the extract⁵

The Shade dried & powdered leaves (400 gm.) were subjected to continuous hot extraction with methanol in Soxhlet extractor for 48 hrs, followed by concentrating extract under vacuum. The extracts were stored in airtight bottle until use.

4 Phytochemical studies

The Methanolic extract of *Bambusa arundinacea* (BA) was subjected to phytochemical analysis. Methanolic extract showed the presence of flavonoids, saponins, carbohydrates, and terpenoids.

5 Induction of CFA and BA treatment

Adult wistar female rat with an initial body weight of 200 to 300g were taken, and divided into six groups each

containing six animals. On day zero, all rats were injected into the sub plantar region of the left hind paw with 0.1ml of Complete Freund's Adjuvant. This consist of Mycobacterium butyricum suspended in heavy paraffin oil by thorough grinding with motor and pestle to give a concentration of 6mg/ml. Dosing with the test and standard compounds was started on the zero day and continued for 12 days according to the following schedule: Group I: Normal control (Distilled water), Group II: Disease control (suspension of 1% CMC), Group III: Dexamethasone (5 mg/kg, i.p. standard), Group IV: Methanolic extract of *Bambusa arundinacea* (100 mg/kg, p.o.), Group V: Methanolic extract of *Bambusa arundinacea* (200 mg/kg, p.o.), Group VI: Methanolic extract of *Bambusa arundinacea* (300 mg/kg, p.o.). From day 13th to 21st, the animals were not dosed with the test compound or the standard. The following parameters were measured¹⁰.

5.1 Evaluation of the development of Arthritis

Rats were inspected daily for the onset of arthritis characterized by edema in the paws. The incidence and severity of arthritis were evaluated using a system of arthritic scoring, and measurement of bi-hind paw volumes every 3 days beginning on the day when arthritic signs were first visible. Animals were observed for presence or absence of nodules in different organs like ear, fore paw, hind paw, nose and tail. Animal were score 0 for absence and 1 for presence of nodules. 5 was the potential maximum of combined arthritic score per animal. Hind paw volume was measured using plethysmometer. Paw volumes of both hind limbs were recorded from day of Treatment started to 21st day at three day interval using mercury column plethysmometer.¹¹

5.2 Rheumatoid Factor

The latex turbidimetry method was used in the present study using RF turbilatex kit of SPINREACT Company. Calibration was carried out for linear range up to 100 IU/ml. The reading of RF factor of all the groups obtained was compared with the control animals and was expressed as IU/ml RF.¹²

5.3 Radiography

Female wistar rats were sacrificed on 21st day of CFA administration and legs were removed and placed on formalin containing plastic bags. This plastic bag was kept at a distance of 90 cm from the X-ray source and Radiographic analysis of arthritic and treated animal hind paw were performed by X-ray machine with a 300-mA exposition for 0.01 s. An investigator blinded for the treatment regimen performed radiograph score. The following radiograph criteria were considered: These scores (destroyed or intact joint) were used as a quantal test for bone necrosis. Radiographs were carefully examined using a stereo microscope and abnormalities were graded as follows:

- (i) Periosteal reaction, 0 - 3 (None, Slight, Moderate, Marked);
- (ii) Erosions, 0 - 3 (None, Few, Many Small, Many Large);

- (iii) Joint space narrowing, 0 - 3 (None, Minimal, Moderate, Marked);
- (iv) Joint space destruction, 0 - 3 (None, Minimal, Extensive, Ankylosis).

Bone destruction was scored on the patella as described previously.¹³

5.4 Effect on Spleen-Index

At the end of the experiment, after sampled for serum, all mice were sacrificed by ether anesthesia. All the spleens of mice were weighed immediately after dissection. The spleen indexes were calculated by using the following formula:¹⁴

$$\text{Index} = \frac{\text{Spleen weight of CFA rat} / \text{Body weight of CFA rat}}{\text{Spleen weight of normal rat} / \text{Body weight of normal rat}}$$

5.5 Histological Processing and Assessment of Arthritis Damage

Rats were sacrificed by ether anesthesia. Knee joints were removed and fixed for 4 days in 4% formaldehyde. After decalcification in 5 % formic acid, the specimens were processed for paraffin embedding tissue sections (7 µm thick) and were stained with haematoxylin and eosin, or safranin. An experienced pathologist, unaware of the different drug treatments scored the condition of tibiotarsal joints. Histopathological changes were scored using the following parameters. Infiltration of cells was scored on a scale from 0 to 3, depending on the amount of inflammatory cells in the synovial tissues. Inflammatory cells in the joint cavity were graded on a scale from 0 to 3 and expressed as exudate. A characteristic parameter in arthritis is the progressive loss of articular cartilage. This destruction was separately graded on a scale from 0 to 3, ranging from the appearance of dead chondrocytes (empty lacunae) to complete loss of the articular cartilage. Bone erosion was scored on a scale ranging from 0 to 3, ranging from no abnormalities to complete loss of cortical and trabecular bone of the femoral head. Cartilage and bone destruction by pannus formation was scored ranging from 0, no change; 1, mild change (pannus invasion within cartilage); 2, moderate change (pannus invasion into cartilage/subchondral bone); 3, severe change (pannus invasion into the subchondral bone); and vascularity (0, almost no blood vessels; 1, a few blood vessels; 2, some blood vessels; 3, many blood vessels). Histopathological changes in the knee joints were scored in the femur region on 5 semi-serial sections of the joint, spaced 70 µm apart. Scoring was performed on decoded slides by two observers, as described earlier.¹⁵⁻¹⁸

RESULT & DISCUSSION

The hind paw injected with Complete Freund's Adjuvant became gradually swollen and reached its peak at 21st day. Figure-1 showed the results obtained for the different doses of BA and the standard drug (Dexamethasone 5mg/kg) in the Complete Freund's Adjuvant-induced (CFA) paw edema test at specific time intervals. It was obvious that during 21st day treatment paw edema in

disease control inflamed paw is increase in time dependent manner and all administration groups significantly

inhibited the development of joint swelling induced by Complete Freund's Adjuvant.

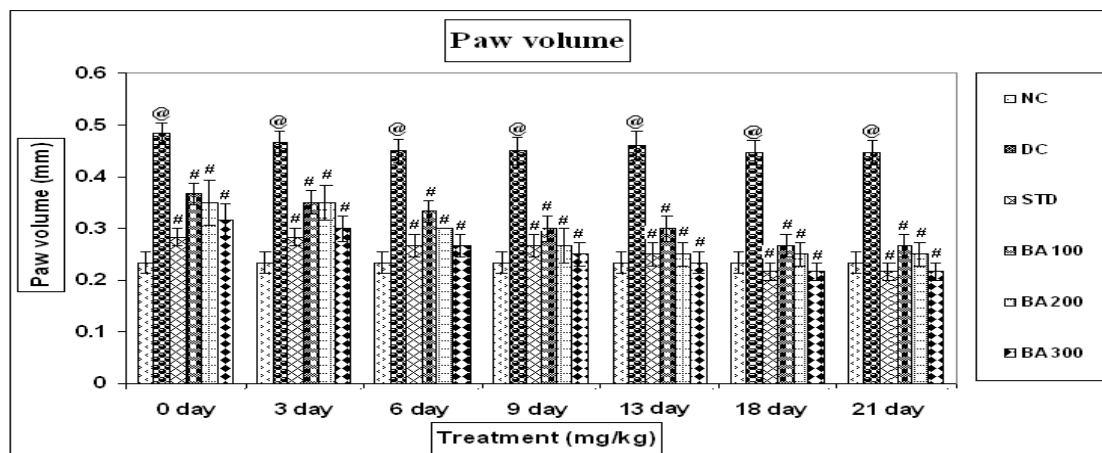


FIGURE 1: Effect of Methanolic extract of *Bambusa arundinacea* on Paw Edema in Arthritic Rats

Arthritic index and Rheumatoid factor were significantly decreased in treatment with BA (100 mg/kg, 200 mg/kg and 300mg/kg) and Dexamethasone (5 mg/kg) treated animal as compared to disease control treatment as shown in Figure 2, Figure 3.

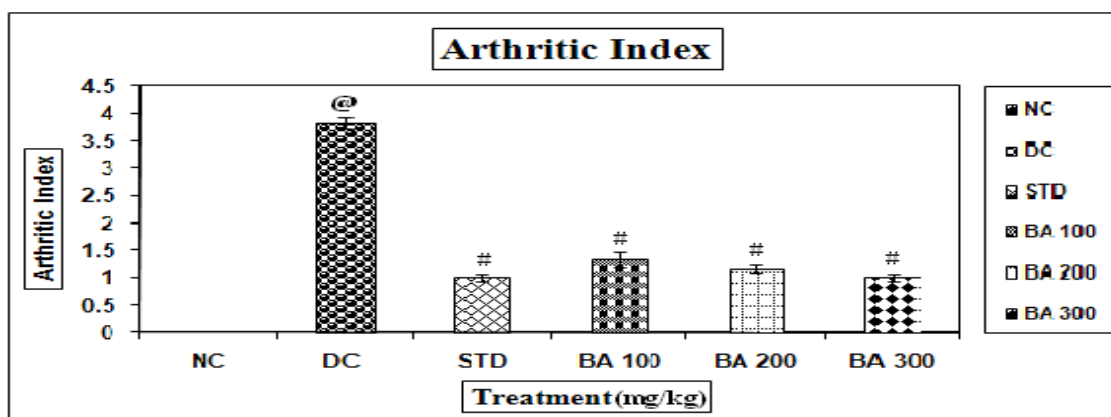


FIGURE 2: Effect of Methanolic extract of *Bambusa arundinacea* on Arthritic index in Arthritic Rats

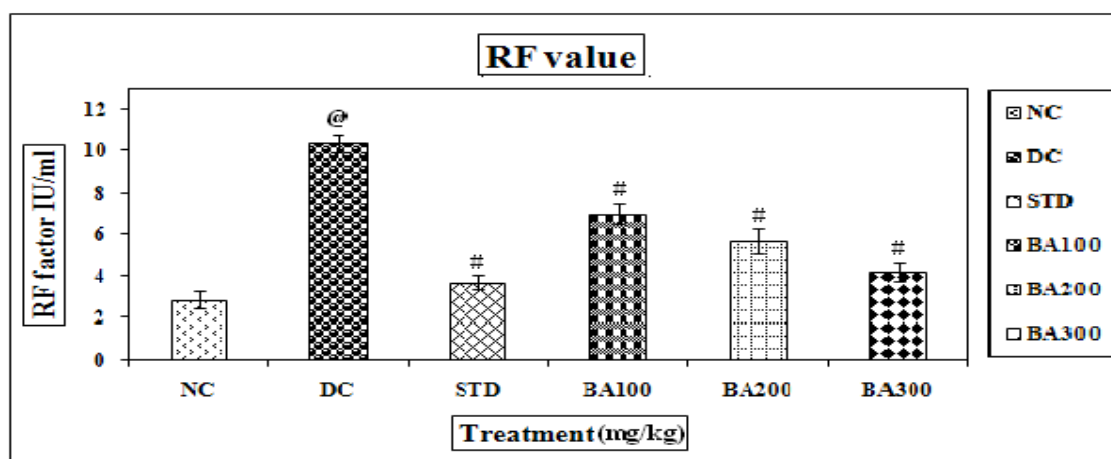


FIGURE 3: Effect of Methanolic extract of *Bambusa arundinacea* on RF in Arthritic Rats

Bone destruction, which is a common feature of adjuvant arthritis, was examined by radiological analysis. Complete Freund's Adjuvant treated rats had developed definite joint space narrowing of the intertarsal joints, diffuse soft tissue swelling that included the digits, diffuse demineralization of bone, marked periosteal thickening, and cystic enlargement of bone and extensive erosions produced narrowing or pseudo widening of all joint spaces. In contrast, in rats treated with BA attenuate abnormalities consisted of asymmetric soft tissue swelling and small erosions, periosteal thickening, and minimal joint space narrowing, predominantly localized to the proximal areas of the paws as shown in Figure 4.

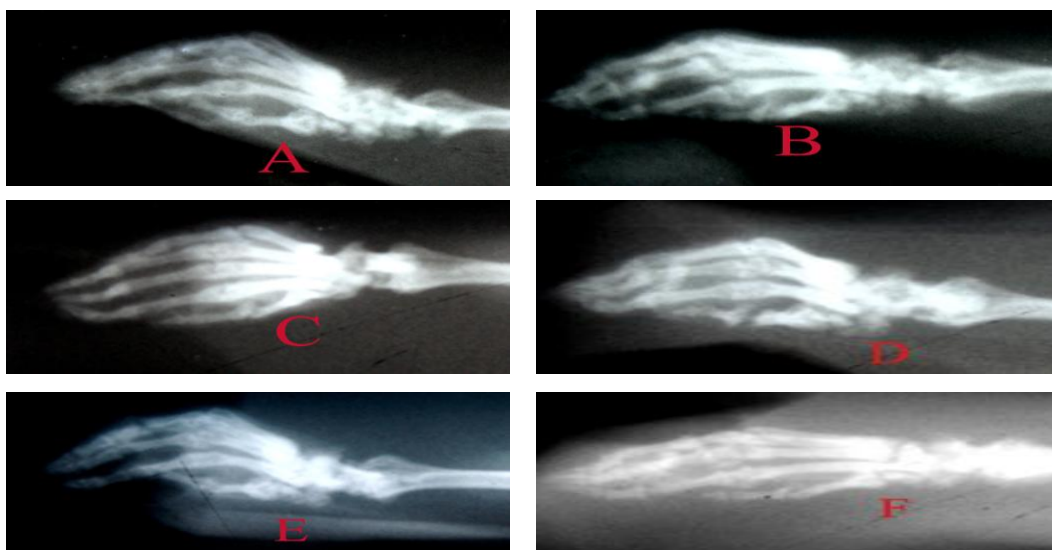


Figure 4: Effect of Methanolic extract of *Bambusa arundinacea* on Radiographs of tibiotarsal joints.

A- Normal Control, B- Disease Control, C- Dexamethasone,
D- BA 100 mg/kg, E- BA 200 mg/kg, F- BA 300 mg/kg

Despite a similar clinical course of arthritis, disease control rats suffered from more pronounced bone destruction than *Bambusa arundinacea* treated animals as seen on radiographs taken on day 21st day in complete Freund's adjuvant induce arthritis. BA treated animals had a more pronounced decrease in bone density, destruction of bony structures, as compare to Disease control as shown in Figure 5. Abrogation of disease progression by BA was further supported by the histopathologic analysis of the

joints from these animals. Rats that had been prophylactically treated with BA at the time CFA immunization showed no histological abnormalities, with no evidence of cartilage erosion in their joints in contrast to the diseased control rats that displayed completely destroyed joint architecture as shown in Figure 6. CFA-induced arthritic scores were reduced across all disease parameters assessed (inflammation, cartilage damage).

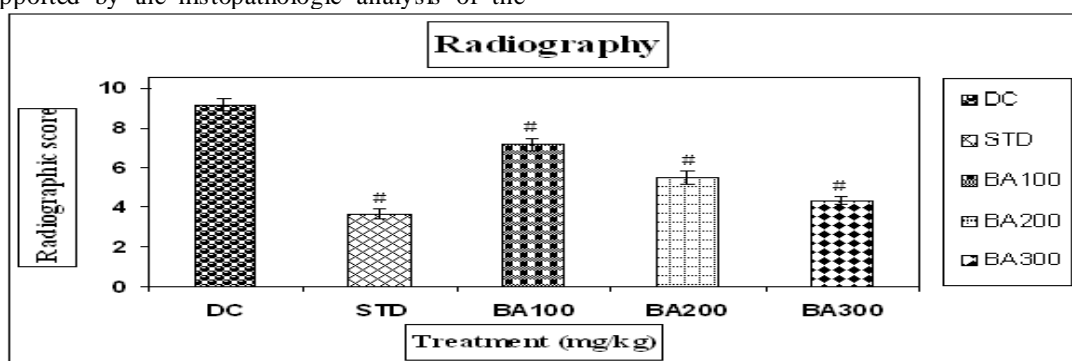


FIGURE 5: Effect of Methanolic extract of *Bambusa arundinacea* on Radiographic score in Arthritic Rats

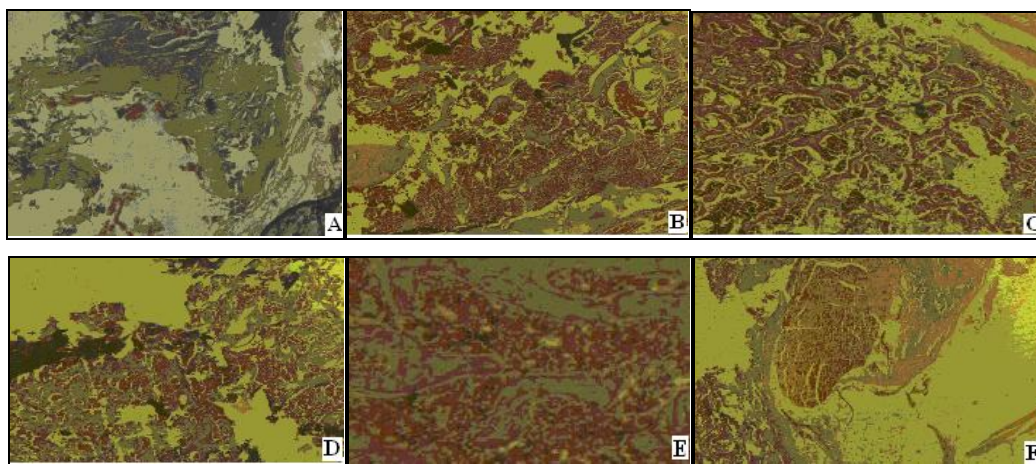


Figure 6: Histopathology of joints indicates treatment with Methanolic extract of *Bambusa arundinacea* prevent bone erosion.

A- Normal Control, B- Disease Control, C- Dexamethasone,
D- BA 100 mg/kg, E- BA 200 mg/kg, F- BA 300 mg/kg

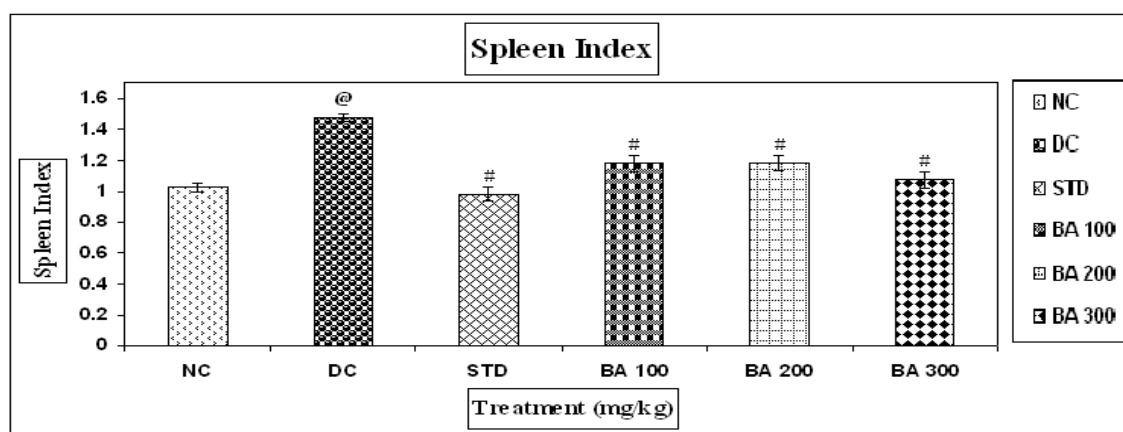


FIGURE 7: Effect of Methanolic extract of *Bambusa arundinacea* on Spleen index in Arthritic Rats

The spleen provided a readily available source of cells known to be involved in arthritis. Increased cellularity in the spleen of arthritic rats engendered interest as to the potential for concomitant classical antibody formation. BA inhibits splenomegaly, which can enhance inhibitory effect of drug as shown in Figure 7. It may be that in vivo BA gives the chronic anti-inflammatory effect by suppression of splenic lymphocytes, which might then result in reduced immunological activation, and subsequent inhibition of the infiltration of circulating lymphocytes into the synovium.

CONCLUSION

Our data suggested that *Bambusa arundinacea* possesses significant Anti-arthritis activity. The possible mode of antiarthritic activity of Methanolic extract of *Bambusa arundinacea* appears to be, Possessing anti-inflammatory activity showed in arthritic parameters like Paw edema,

Arthritic index, Rheumatoid factor, improving bone erosion. All these results thus predict that the drug provide pharmacological rationale for the traditional use of the drug against inflammatory disorders such as rheumatoid arthritis.

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CONFLICTS OF INTEREST: There is no conflict of interest.

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